

Amendment to the Specification

At page 2, paragraph [09], amend the application as follows:

[09] Provided herein are methods for the purification of a protein of interest from its fusion analog by hydrophobic charge induction chromatography. In one embodiment the method further comprises size exclusion chromatography. In a further embodiment, the method comprises purifying a protein of interest from its fusion analog, comprising: obtaining a protein solution comprising the protein of interest and its fusion analog; adjusting the pH and/or ionic strength of the protein solution with an appropriate buffer for a Hydrophobic Charge Induction Chromatograph (HCIC) resin; contacting the protein solution with an HCIC resin column to allow binding of the protein of interest and its fusion analog to the resin; washing the HCIC resin with an appropriate buffer; and eluting the protein of interest from the HCIC resin by a pH gradient; wherein said protein of interest is substantially free of its fusion analog. In yet a further embodiment, the method comprises purifying an immunoglobulin comprising: obtaining a protein solution comprising the immunoglobulin; adjusting the pH and/or ionic strength of the protein solution with an appropriate buffer for a HCIC resin; contacting the protein solution with the HCIC resin to allow binding of the immunoglobulin to the resin; washing the HCIC resin with an appropriate buffer; and eluting the immunoglobulin from the HCIC resin by a pH gradient, wherein said pH gradient is incrementally decreased and the immunoglobulin is a F(ab')₂ fragment and/or a Fab' fragment and said immunoglobulin is substantially free of other proteins.

At page 4, amend paragraph [13] – Fig. 1 under the section of “Brief Description of the Drawings” as follows:

[13] Figure 1 is a diagram of a fully assembled, mature monoclonal antibody (A), an antibody fragment (B) and their respective fusion analogs (C and D).

Glucoamylase (GA) ~~is shown in pink~~ is represented as large grained ovals. The ~~red stippled~~ diamonds on the Fc portion of the mature immunoglobulin represent glycosylation sites. It should be appreciated that the number of GA moieties on the fusion analogs may vary from that shown due to cellular processing. The chains, although produced as fusions, may undergo proteolytic processing, either intracellularly or extracellularly, to remove a GA moiety. An immunoglobulin chain can be covalently attached to one or two other chains through a cystine linkage. In the case of F(ab')₂, two Fab units are attached in this way to form the dimer.

At page 9, paragraph [39], amend the application as follows:

[39] Preferred culture conditions for a given cell line may be found in the scientific literature and/or from the source of the cell line such as American Type Culture Collection (ATCC; "<http://www.atcc.org/>" P.O Box 1549, Manassas VA 20108). Typically, after cell growth has been established, the cells are exposed to conditions effective to cause or inhibit the expression of the protein of interest and/or their fusion analogs.

At page 14, paragraph [69], amend the application as follows:

[69] The peptide of interest is eluted from the column by a pH gradient. In some embodiments, the pH gradient begins at a pH of about 8.0 and ends at a pH of about 2.5. In other embodiments the pH gradient begins at a pH of about 2.5 and ends at a pH of about 8. In further embodiments, the pH gradient is a step pH gradient and comprises between 2 and 6 steps. The rate at which the desired protein is desorbed from the resin is determined by hydrophobic and charge characteristics of the protein of interest. The proteins will elute in order of increasing hydrophobicity. To a limited extent, the flow rate will affect the elution profile with faster flow rates giving broader peaks. Appropriate buffers are, for example, Elution Buffer #1:

100mM Sodium Acetate, pH 5.6; Elution Buffer #2: 100mM Sodium Acetate, pH 4.75; Elution Buffer #3: 100mM Sodium Acetate, pH 4.00; and Elution Buffer #4: 100mM Sodium Citrate, pH 2.5.

At page 23, "Abstract of the Disclosure" paragraph [107], amend the application as follows:

[107] ~~Described herein are methods for purifying a protein of interest from a mixture of proteins wherein the mixture comprises the protein of interest and a fusion analog thereof. The method begins with a recovery step in which filtration is used to remove cells and concentrate the protein of interest. Liquid chromatography is then used to remove fusion analogs and other contaminants resulting in a composition substantially free of contaminants. Substantially pure compositions of the protein of interest find use in therapeutics preparations.~~ The present invention relates to a method of purifying a protein of interest from its fusion analog, comprising obtaining a protein solution comprising a protein of interest and its fusion analog; adjusting the pH and/or ionic strength of the protein solution with an appropriate buffer for use with a Hydrophobic Charge Induction Chromatograph (HCIC) resin, contacting the protein solution and HCIC resin column to allow binding of the protein of interest and its fusion analog, washing the resin and eluting the protein of interest from the resin by a pH gradient; wherein the protein of interest is substantially.

Please substitute the attached replacement drawings (Figures 1A – 9, 14 sheets) for the original drawings (Figures 1A – 9, 13 sheets).